## Nitric Oxide: Genomic Instability And Synthetic Lethality

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Regardless of etiology, inflammatory conditions are characterized by overexpression of inducible nitric oxide synthase (iNOS) and overproduction of nitric oxide and reactive nitrogen species (NO/RNS) in epithelial and inflammatory cells at the site of carcinogenesis.

NO/RNS produced in inflamed tissues can contribute to the process of carcinogenesis by different mechanisms. One of these mechanisms is NO-dependent stimulation of genomic instability by inhibiting of Breast Cancer type 1 Susceptibility protein (BRCA1) expression. Block of BRCA1 expression shifts DNA double-strand breaks (DSB) repair from error-free high-fidelity homologous recombination repair (HRR) to error-prone nonhomologous end joining (NHEJ).

BRCA1 epigenetically block miRNA-155 expression via its association with HDAC2, which deacetylates histones H2A and H3 on the miRNA-155 promoter. The miRNA-155 is responsible for post-translational silencing of essential members of mismatch repair (MMR) core: MSH2, MSH6, and MLH1 proteins. They epigenetic inactivation induces DNA microsatellite instability (MSI). Recently, we demonstrated NO-dependent downregulation of MMR core proteins (MSH2, MSH6, and MLH1) through the ↓BRCA1/↑miRNA-155 signaling pathway. Hence, another NO-dependent mechanism of genomic instability is downregulation of MMR core proteins and stimulation of the DNA MSI.

Loss or inhibition of Poly(ADP-ribose) polymerase 1 (PARP1) activity results in accumulation of DNA single-strand breaks, which are subsequently converted to DSB by the transcription machinery. In BRCA-positive cells, DSB are repaired by HRR, but they cannot be properly repaired in BRCA1-deficient cells, leading to genomic instability, chromosomal rearrangements, and cell death. Our data demonstrated that combination of NO-donors with PARP inhibitors significantly sensitized the BRCA1-positive cancer cells to DNA-damaging agents.

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# Young Investigation Session Selected Oral Communications

## Activation Of Wild-Type Hras Suppresses The Earliest Stages Of Pancreatic Cancer

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*Background:* The RAS family of small GTPases is comprised of *HRAS*, *NRAS,* and *KRAS. KRAS* is invariably oncogenically mutated in pancreatic cancers, which is known to induce this disease. Beyond oncogenic KRAS, redox-dependent reactions have been implicated in the activation of the remaining wild-type RAS proteins in pancreatic cancer cell lines. These results suggest a possible involvement of wild-type RAS proteins in pancreatic cancer.

*Aims:* To evaluate the impact of genetically suppressing wild-type RAS expression on pancreatic cancer.

*Methods: Hras* homozygous null mice (*Hras*$^{-/-}$) were crossed into a *Pdx-Cre; LSL-Kras*$^{G12D/+}$ (*KC*) murine background in which oncogenic Kras is activated in the pancreas to promote preinvasive pancreatic cancer. Tumor burden was then measured at different stages of disease.

*Results: HRas*$^{-/-}$;*KC* mice exhibited more precancerous lesions in the pancreas and more off-target skin papillomas compared to their wild-type counterparts, suggesting that Hras suppresses early oncogenic Kras-driven tumorigenesis, possibly at the time of initiation. Loss of *Hras* also reduced the survival of mice engineered to develop aggressive pancreatic cancer by the additional disruption of one allele of the tumor suppressor p53 (Trp53$^{R172H/+}$). However, this survival advantage was lost when both alleles of *Trp53* were mutated, suggesting that wild-type Hras inhibits tumorigenesis in a p53-dependent fashion.

*Conclusions:* Loss of wild-type Hras promotes the earliest stages of pancreatic tumorigenesis, and moreover results in more rapid progression of the disease. As such, mechanisms leading to activation of wild-type Ras proteins, including but not limited to redox-dependent reactions, may influence the development of pancreatic cancer.

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## Biochemical And Tumorigenic Effects Of Redox Modification Of Ras-G12c By Nitric Oxide

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*Background:* The Ras family of small GTPases cycle between an inactive, GDP-bound state and an active, GTP-bound state. When bound to GTP, Ras engages and activates a number of effectors that mediate proliferative and survival signals. Ras is mutated in over 30% of human cancers, usually at codons 12, 13, or 61, to remain in this active, GTP-bound state, which promotes tumorigenesis. One of these oncogenic mutations that commonly occurs in lung cancer is G12C. Recently, it was shown that alkylating agents that react with the thiol functional group of this mutant amino acid can inactivate oncogenic Ras$^{G12C}$.

*Aims:* Given that Cys$^{12}$ of Ras$^{G12C}$ is accessible to thiol alkylating agents and forms interactions within the electrostatic phosphoryl-binding loop of Ras, we postulated that Cys$^{12}$ may possess an altered p*K*a, potentially allowing this residue to be modified by NO and other cellular oxidants.

*Methods:* We conducted several biochemical analyses to determine whether nitrosylation of Ras$^{G12C}$ alters its activity and structure *in vitro*. We also determined the biological effects of increasing NO production on the tumorigenic growth of cells transformed by Ras$^{G12C}$.

*Results:* We found that Cys$^{12}$ has a depressed p*K*a of 7.4, which increases the susceptibility of the thiol to modification by oxidation or nitrosylation at physiological pH. We also found that coexpressing active eNOS$^{S1177D}$ and Ras$^{G12C}$ accelerated tumorigenic growth of human and murine cell line xenografts.

*Conclusion:* Modification of Cys$^{12}$ in mutant, oncogenic Ras$^{G12C}$ may promote its tumorigenic activity.

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